

© Nouioui, I., Klenk, H.P., Igual, J.M., Gulvik, C.A., Lasker, B.A., McQuiston, J.R. 2019. The definitive peer reviewed, edited version of this article is published in International Journal of Systematic and Evolutionary Microbiology, 69, 4, 2019, <http://dx.doi.org/10.1099/ijsem.0.003267>

Streptacidiphilus bronchialis* sp. nov., a ciprofloxacin resistant isolate from a human clinical specimen; reclassification of *Streptomyces griseoplanus* as *Streptacidiphilus griseoplanus* comb. nov. and emended description of the genus *Streptacidiphilus

Imen Nouioui¹, Hans-Peter Klenk¹, José Mariano Igual², Christopher A. Gulvik³, Brent A. Lasker³ and John R. McQuiston³

¹School of Natural and Environmental Sciences, Ridley Building 2, Newcastle University, Newcastle upon Tyne, NE1 7RU

²Instituto de Recursos Naturales y Agrobiología de Salamanca, Consejo Superior de Investigaciones Científicas (IRNASA-CSIC), c/Cordel de Merinas 40-52, 37008 Salamanca, Spain

³Bacterial Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Disease, Centers for Diseases Control and Prevention, Atlanta, GA 30333

Section: Actinobacteria

Key words Actinobacteria, polyphasic taxonomy, phenotyping, bronchial lavage

Correspondence: Brent A. Lasker, Bacterial Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Disease, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333. Email: blasker@cdc.gov; Phone: 404-639-3905

The GenBank accession number for the 16S rRNA gene and genome sequence for *Streptacidiphilus bronchialis* strain 15-057A^T is KR346911 and PXZ101000000.1, respectively.

Abstract

The taxonomic position of strain 15-057A^T, an acidophilic actinobacterium isolated from the bronchial lavage of an 80 year old male, was determined using a polyphasic approach incorporating morphologic, phenotypic, chemotaxonomic and genomic analyses. Pairwise 16S rRNA gene sequence similarities calculated using the GGDC web server between strain 15-057A^T and its close phylogenetic neighbours, *Streptomyces griseoplanus* NBRC 12779^T and *Streptacidiphilus oryzae* TH49^T were 99.7% and 97.6%, respectively. The G+C content of isolate 15-057A^T was determined to be 72.6 mol %. DNA-DNA relatedness and average nucleotide identity between isolate 15-057A^T and *S. griseoplanus* DSM 4009^T were 29.2% +/-2.5% and 85.97%, respectively. Chemotaxonomic features of isolate 15-057A^T were consistent with its assignment within the genus *Streptacidiphilus*: whole cell hydrolysate contained LL-A₂pm as diagnostic diamino acid and glucose, mannose and ribose as cell wall sugars; the major menaquinone is MK9(H₈); the polar lipids profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, glycerophospholipid, aminoglycerophospholipid and unknown lipid; the major fatty acids (>15%) are *anteiso*-C_{15:0} and *iso*-C_{16:0}. Phenotypic and morphological

traits distinguish isolate 15-057A^T from its closest phylogenetic neighbours. The results of our taxonomic analyses showed that strain 15-057A^T represents a novel species within the evolutionary radiation of the genus *Streptacidiphilus* for which the name *Streptacidiphilus bronchialis* sp. nov. is proposed, with strain 15-057A^T (= DSM 106435^T = ATCC BAA-2934) as a type strain.

The genus *Streptacidiphilus* of the family *Streptomycetaceae* [1] was established by Kim *et al.* [2] and currently comprised of twelve species with validly published names: *Streptacidiphilus albus* [2], *Streptacidiphilus anmyonensis* [3], *Streptacidiphilus carbonis* [2], *Streptacidiphilus durhamensis* [4], *Streptacidiphilus hamsterleyensis* [5], *Streptacidiphilus jiangxiensis* [6], *Streptacidiphilus melanogenes* [3], *Streptacidiphilus monticola* [7], *Streptacidiphilus neutrinimicus* [2], *Streptacidiphilus oryzae* [8], *Streptacidiphilus rugosus* [3] and *Streptacidiphilus toruniensis* [9]. Members of the genus *Streptacidiphilus* share many morphologic and phenotypic properties with the genus *Streptomyces*. *Streptacidiphilus* strains are Gram-positive, aerobic, chemoorganotrophs acidophilic actinomyces which produce branched mycelium with aerial hyphae, long chains of spores and growth at pH 3.5 to 6 [2]. Representatives of this genus have been isolated primarily from acidic soils such as coniferous soils which explains their significant ecological role in the organic matter turnover [10,11] and they are well known by their ability to produce antifungal compounds [12], chitinases [13] and diastases [14]. In this present report, strain 15-057A^T was recovered from a bronchial lavage of an 80 year old male and analyzed in the Special Bacteriology Reference Laboratory (SBRL) at the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA. The taxonomic status of this isolate was examined using a polyphasic approach. Based on the morphologic, phenotypic, taxonomic and genotypic results, the isolate was assigned to a novel species within the genus *Streptacidiphilus*.

Strain 15-057A^T was isolated from the bronchial wash of an 80 years old male patient from the state of Tennessee. The isolate was plated on Trypticase soy agar (TSA) supplemented with 5 % sheep blood and incubated aerobically at 35°C for 3 – 5 days. Identification of the test strain was initially determined by the SBRL at CDC by 16S rRNA gene analysis. The test strain 15-057A^T, together with the reference strain *Streptomyces griseoplanus* DSM 40009^T [15], obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) were maintained in International *Streptomyces* Project (ISP-2) medium and as suspension of cells in 20%v/v glycerol at -80° C. Morphological and cultural characteristics of strain 15-057A^T were examined in presence of different media; TSA supplemented with 5% sheep blood, heart infusion agar (HIA) supplemented with 5% rabbit blood, Middlebrook 7H11 (MB7H11) agar slants, potato dextrose (PD) agar, Nutrient agar (NA), ISP2, ISP3, ISP6, ISP7, GYM, N-Z-Amine and Sucrose Bennett's agar plates [16] after incubation for 7 days at 37°C. The ability of the studied strain to grow at different temperature 20, 25, 30, 35, 40, 45 and 50°C in the presence of ISP 2 medium was carried out as previously described by Berd [17].

Freeze-dried biomass of strain 15-057^T and *S. griseoplanus* DSM 40009^T was obtained from culture prepared in ISP2 broth medium shaken at 180 revolutions per minute (rpm) for 5 days at 37°C and 28°C, respectively; harvested cells were washed twice with distilled water. Fatty acid analysis was determined from wet biomass prepared under the same condition cited above. Standard chromatographic procedures were used to determine the isomers of A₂pm [18], cell wall sugars [19], isoprenoid quinones [20] and polar lipids [21] as modified by Kroppenstedt and Goodfellow [22]. Fatty acids extraction were carried out following the protocol of Miller [23] as modified by Kuykendall *et al.* [24]. The extracts were analyzed using gas chromatography (Agilent 6890N instrument) and peak identifications were performed based on the Microbial Identification System (MIDI, Inc Sherlock version 6.1) [25]. Chemotaxonomic features of

the strain 15-057A^T were consistent with the genus *Streptacidiphilus*. Whole cell hydrolysates contained LL-A₂pm as diaminopimelic acid and glucose, mannose and ribose as cell wall sugars; polar lipid pattern consisted of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), glycerophospholipid (GPL), unknown lipid (L) and aminoglycolipid (AGL) (Figure S1); predominant isoprenologue (>25%) was found to be MK9(H₈). Major fatty acid profile (>15%) are *anteiso*-C_{15:0} and *iso*-C_{16:0} (Table S1). The type strain of *S. griseoplanus* was characterised by LL- A₂pm in the peptidoglycan layer and glucose, mannose (trace) and ribose as cell wall sugars. It has MK9(H₆) as predominant menaquinone; *anteiso*-C_{15:0} and C_{16:0} as major fatty acid (15%) (Table S1) and more complex polar lipid profile consisted of DPG, PI, PE, GPL, AGL, glycolipid (GL), aminolipid (AL) and three unknown phospholipids (PL₁₋₃) (Figure S1). The fatty acid, DAP, menaquinone and the polar lipids patterns of these strains are in line with what has been described for the genus *Streptacidiphilus* [26] and for *S. oryzae* TH49^T which found to be the second nearest phylogenetic neighbour to the studied strain (Table 2). However, the results of the cell wall sugars analyses of strains 15-057A^T and DSM 4009^T (glucose, mannose and ribose) are not in line with those described for *Streptacidiphilus* genus (galactose and rhamnose) and for *S. oryzae* (galactose, glucose, mannose, ribose) (Table 2). Strain 15-057A^T showed distinguishable chemotaxonomic traits not only from its nearest neighbour strain DSM 4009^T, but also from its close relatives retrieved in the EzTaxon database: *Kitasatospora arboriphila* [27], *Kitasatospora aureofaciens* [28-29], *Streptomyces avellaneus* [30], *Kitasatospora viridis* [31] and *Kitasatospora kifunensis* [32] (Table 2).

Strain 15-057A^T and *Streptomyces griseoplanus* DSM 4009^T were tested with a broad range of carbon and nitrogen sources and for their ability to grow in the presence of inhibitory compounds using GENIII microplates in an Omnilog device (Biolog) for 7 days at 37°C. The resultant data were analyzed using opm R package version 1.3.36 [33, 34]. Degradation and hydrolysis tests of casein, cellulose, chitin, 0.3%

elastin, gelatin, guanine, 0.4% hypoxanthine, nitrate reduction, pectin, starch, 1% tributin, tween 20, 40, 60 and 80, urea, 4% xanthine and 0.4% xylan were performed according to the methods described by Berd, [17] and Weyant *et al.* [35]. Gram- and modified Kinyon acid-fast staining was performed using the methods described by Berd [17].

Isolate 15-057A^T was found to be aerobic, Gram-stain positive, non-motile and acid-fast negative. Colonies cultured on TSA supplemented with 5% sheep blood were elevated with irregular and lobate edges, white to light gray and no hemolysis. Hemolysis was observed following growth on HIA medium with 5% rabbit blood after growth for 5 days at 37°C. Colonies were observed to be circular and raised, with shiny to pale grey white and cottony with aerial mycelium in MB7H11 agar slants. However, they acquired pale green color with white margins in presence of PD plate. After 7 days of incubation at 37°C, the test strain developed a grey aerial mycelium on GYM, ISP 2 and ISP 3 medium while aerial mycelium acquired a beige color on TSA plates and white on ISP 7, N-Z Amine agar and sucrose Bennett's agar plates. The test strain was not able to grow in the presence of nutrient agar medium or to produce aerial mycelium in ISP 6 agar plates unlike its nearest neighbor *Streptomyces griseoplanus* DSM 40009^T (Table 1). Growth of the test strain was observed after 5 – 7 days of incubation in ISP 2 agar plate at a range of temperatures from 20 to 40°C but not at 45°C. Isolate 15-057A^T unlike *S. griseoplanus* DSM 40009^T is able to degrade starch, elastin, guanine and reduce nitrate. Likewise, isolate 15-057A^T unlike *S. griseoplanus* DSM 40009^T is able to utilize acetic acid, L-alanine, D-arabitol, L-arginine, butyric acid, D-glucose-6-phosphate, α -keto-glutaric acid, L-histidine, inosine, D-malic acid, pectin, propionic acid, D-serine, bromo-succinic acid, sucrose and sodium lactate. More phenotypic properties that distinguishing isolate 15-057A^T from its closest phylogenetic neighbour, *Streptomyces griseoplanus* DSM 40009^T are shown in Tables 1 and 2.

Additional antibiotic resistance tests were carried out for the test strain. Minimum inhibitory concentrations (MICs) for amikacin, ampicillin, ceftriaxone, ciprofloxacin, clarithromycin, minocycline, trimethoprim/sulfamethoxazole, imipenem, amoxicillin/clavulanate, moxifloxacin, linezolid and vancomycin were performed and assessed according to the breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) (NCCLS, 2003). Commercial antimicrobial panels were provided from PML Microbiologicals, Inc. Isolate 15-057A^T was susceptible to amikacin, ampicillin, ceftriaxone, clarithromycin, minocycline, trimethoprim/sulfamethoxazole, imipenem, amoxicillin/clavulanate, moxifloxacin, linezolid and vancomycin, but resistant to ciprofloxacin.

Genomic DNA extraction and purification for PCR-mediated amplification of a 1439-bp 16S rRNA gene fragment and DNA sequencing were performed as described by Lasker *et al.* [36]. 16S rRNA gene sequence of isolate 15-057A^T was compared with the corresponding sequence of the nearest type strain by EzBioCloud (<http://www.ezbiocloud.net>. [37]). Pairwise sequence similarities were calculated using the method recommended by Meir-Kolthoff *et al.* [38] for 16S rRNA gene sequences available via the Genome-to-Genome Distance Calculator (GGDC) web server [39] at <http://ggdc.dsmz.de/> using DSMZ phylogenetic pipeline [40] adapted to single genes. A multiple sequence alignment was created with MUSCLE [41]. Maximum-likelihood (ML) [42] and maximum parsimony (MP) [43] trees were inferred from the alignment with RAxML [44] and TNT [45], respectively. For ML, rapid bootstrapping in conjunction with the autoMRE bootstrapping criterion [46] and subsequent search for the best tree was used; for MP, 1,000 bootstrapping replicates were used in conjunction with tree-bisection-and-reconnection branch swapping and ten random sequence addition replicates. The sequences were checked for a compositional bias using X² test as implemented in PAUP* [47]. Neighbor-joining phylogenetic trees [48] was constructed using MEGA7.0.14 software package [49].

BLAST analysis of the almost complete 16S rRNA gene sequence of strain 15-057A^T, using the EzTaxon database, showed 99.5% and 97.5% - 97.8% sequence similarity with the type strains of *S. griseoplanus* and *K. arboriphila*, *K. aureofaciens*, *S. oryzae*, *S. avellaneus*, *K. kifunensis* and *K. viridis*, respectively. These similarities were partially in concordance with the topology of the phylogenetic tree because only *S. griseoplanus* NBRC 12779^T and *S. oryzae* appeared as the closest neighbours with the studied strain. Strain 15-057A^T formed with *S. griseoplanus* NBRC 12779^T a well-supported subclade closely related to *S. oryzae* TH49^T within the evolutionary radiation of the genus *Streptacidiphilus* as shown in Figure 1. Pairwise 16S rRNA gene sequences similarities calculated using the GGDC web server between strain 15-057A^T strain and its phylogenetic neighbours, *S. griseoplanus* DSM 40009^T and *S. oryzae* TH49^T, are 99.7% and 97.6% with 7 and 36 nucleotides differences, respectively. These later strains were grouped together in a distinct clade divergent from the one accommodating *Streptomyces* and *Kitasatospora* strains including *K. arboriphila* HKI 0189^T, *K. aureofaciens* ATCC 10762^T, *S. avellaneus* NBRC 13451^T, *K. kifunensis* IFO 15206^T and *K. viridis* 52108a^T. In fact, the phylogenetic position of the genus *Streptacidiphilus*, including the *Streptacidiphilus* species cited above, within the phylum *Actinobacteria* have been revised recently based on genome sequences [50]. Thus, the close phylogenetic relatedness of strains 15-057A^T and *S. griseoplanus* DSM 40009^T to *S. oryzae* species highlighted their affiliation to the genus *Streptacidiphilus*.

Kim *et al.* [2] reported on a 23-bp nucleotide sequence unique in the 16S rRNA genes of *Streptacidiphilus* species. Comparison of this 16S rRNA gene signature with the 16S rRNA gene sequence of strain 15-057A^T showed sequence similarity for 19 of 23-bp of this short motif, however, the 23-bp sequences showed 100% sequence similarity with the *Streptomyces* species 16S rRNA gene sequence. Any difference in the 16S rRNA gene signature does not negate the close relatedness of strain 15-057A^T to

Streptacidiphilus taxon as it represents a small fraction of nucleotide information used to investigate the 16S rRNA phylogeny of the studied strain.

Genomic DNA extraction for strain 15-057A^T and *S. griseoplanus* DSM 40009^T for genome sequencing, was performed following the protocol provided by the MoBio Power Microbial Midi DNA Isolation Kit (MoBio Laboratories, Carlsbad, California). Paired-end libraries of genomic DNA were prepared using the NEBNext Ultra DNA library prep kit (New England Biolabs, Inc., Ipswich, MA, USA) and then sequenced on an Illumina MiSeq V2 (2 x 250 bp) platform (Illumina, Inc., San Diego, CA, USA). BBDuk 37.38 (<http://sourceforge.net/projects/bbmap/>) and Trimmomatic 0.36 [51] were used to remove PhiX, clip off adaptors, quality trim, and length filter sequence reads prior to de novo genome assembly in SPAades 3.11.1 [52]. Three sequential rounds of SNP and InDel correction with SAMtools 1.7 [53], bwa mem 0.7.17-r1188 [54], and Pilon 1.22 [55] were used to generate a high fidelity genome assembly. *In-silico* DNA-DNA hybridization was performed between draft genomes of isolate 15-057A^T (7.01 Mbp, 624 contigs, GenBank accession PXZ101000000.1) and *S. griseoplanus* DSM 40009^T draft genome (8.25 Mbp, 802 contigs, GenBank accession LIQR01000000.1) using Formula 2 from the GGDC 2.0 [39] online server. Estimates of the average nucleotide identity between the genome of both strains was determined using the Average Nucleotide Identity (ANI) calculator [56]. The G+C content was determined by the method of Meshah [57].

The ANI value determined between isolate 15-057A^T and *S. griseoplanus* DSM 4009^T was 85.97%, well below the threshold of 95 to 96% value recommended as boundary for prokaryotic species delimitation [58]. Likewise, *in silico* DNA-DNA hybridization was 29.2% +/- 2.5%, a value well below the threshold of 70% proposed by Wayne *et al.* [59] to demarcate genomic species. The genome size of strain 15-057A^T

is 7.01Mb while its nearest phylogenetic neighbours, *S. griseoplanus* DSM 4009^T and *S. oryzae* TH49^T were 8.25Mb and 7.8Mb, respectively. The G+C content of 15-057A^T and *S. griseoplanus* DSM 4009^T were 72.6% and 72.5% respectively, a value slightly above the range of 70 - 72% G+C content for the genus *Streptacidiphilus* [2, 9, 60]. These results call for an emendation of the genus *Streptacidiphilus*.

Interestingly, the 99 amino acid gene *bldB*, involved with morphogenesis, antibiotic production and catabolite control in the core genome of *Streptomyces* species was not detected in the 15-057A^T genome [61]. The *bldB* gene has been noted to be absent in members belonging to the *Streptacidiphilus* and *Kitasatospora* genera [62]. Together, the lack of the 16S rRNA gene *Streptacidiphilus* signature sequence but absence of the *bldB* gene suggests the genome of isolate 15-057A^T may have been subjected to interspecies recombination.

The phenotypic and morphological features of isolate 15-057A^T were consistent with those described for the genus *Streptacidiphilus* [2]. The nearest phylogenetic neighbour of the studied strains, *S. griseoplanus* DSM 4009^T, showed a clear genetic divergence from its supposed genus. The sugar cannot be considered as diagnostic feature of the genus *Streptacidiphilus* since it cannot be used to separate the phylogenetic relatedness of isolate 15-057A^T to the genus *Streptacidiphilus*. This finding is in line with the conclusions highlighted in the recent taxonomic revision of the phylum *Actinobacteria* by Nouioui *et al.* [50].

The results obtained in this polyphasic investigation for isolate 15-057A^T were very interesting since the isolate showed properties of a fuzzy species with genomic and chemotaxonomic properties consistent with members of the *Streptacidiphilus* and *Streptomyces* genera [63]. For instance, the major cell-wall sugars in whole cell hydrolysates were glucose, mannose and ribose and lacked a 23-bp *Streptacidiphilus*

signature sequence indicating more *Streptomyces*-like characters due to partial distortion of the species boundary by interspecies recombination. More importantly, analysis of 16S rRNA gene sequences, whole genome sequence analysis, including lack of the *bldB* gene, and phenotypic differences from its closest phylogenetic neighbor strongly supports the affiliation of the isolate 15-057A^T to the genus *Streptacidiphilus* and to be considered as a new species for which the name *S. bronchialis* sp. nov. is proposed.

The well supported phylogenetic position of the type strain of *S. griseoplanus* within the radiation of *Streptacidiphilus* and its divergence from the representative strains of the genera *Streptomyces* and *Kitasatospora* confirmed the affiliation of *S. griseoplanus* to the genus *Streptacidiphilus*. The misclassification of *S. griseoplanus* species is justified by low number of phenotypic features used at that time for classifying this taxon. Therefore, it is proposed that *S. griseoplanus* be re-classified as *Streptacidiphilus griseoplanus* comb. nov.

Description of *Streptacidiphilus bronchialis* sp. nov.

bronchialis L. pl. n. *bronchia*, the bronchial tubes; L. fem. suff. *-alis*, suffix used with the sense of pertaining to; N.L. masc. adj. *bronchialis*, pertaining to the bronchi, coming from the bronchi.

An aerobic, Gram-stain positive, non-acid fast, nonmotile, streptomycete-like actinomycete. Produces branched mycelium and aerial hyphae. Grows occurs from 20 to 40°C. Forms elevated white to light grey colonies on TSA supplemented with 5% sheep blood and no hemolysis. Colonies are shiny white to pale grey on heart infusion agar with 5% rabbit blood with hemolysis after growth for 5 days at 37°C. Strain developed aerial mycelium acquired different pigmentation depending on the used medium, Grey (GYM, ISP 2 and ISP 3 medium), beige (TSA), white (ISP 7, N-Z-Amine agar and Sucrose Bennett's). The studied strain was not able to grow in presence of nutrient agar medium or to produce aerial mycelium in

ISP 6 agar plates. Grow at pH 5-7 and up to 1% NaCl. The strain was able to metabolise D-arabitol, D-cellobiose, dextrin, D-galactose, β -gentiobiose, D-glucose, D-glucose-6-phosphate, 6, *N*-acetyl-d-glucosamine, L-glutamic acid, L-malic acid, D-maltose, D-mannitol, propionic acid, methyl pyruvate, sucrose, D-trehalose, turanose, sodium bromate as carbon source; L-alanine, L-arginine, l-aspartic acid L-histidine, glycine-proline and D-serine #2 as amino acids; acetic acid, γ -amino-n-Butyric acid, bromo-succinic acid, butyric acid, α - and β -hydroxy-butyric acid, α -keto-butyric acid, α -keto-glutaric acid, D-malic acid, as organic acids; grow in presence of inosine, nalidixic acid, pectin, potassium tellurite and 1% sodium lactate and able to degrade elastin 0.3%, gelatine, guanine, hypoxanthine 0.4%, starch, tween 20, 40, 60, 80, urea. Polar lipids pattern consisted of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), glycopospholipid (GPL), unknown lipid (L) and aminoglycolipid (AGL); whole cell hydrolysates include LL-A₂pm and glucose, mannose and ribose; predominant menaquinone (>25%) was MK9(H₈); major fatty acids (>15%) are *anteiso*-C_{15:0} and *iso*-C_{16:0}. The DNA G+C content of the genome is 72.6 mol %.

The type strain 15-057A^T (=DSM 106435^T = ATCC BAA-2934) was isolated from the bronchial lavage of an 80 year old male patient from the state of Tennessee. The GenBank accession number of the 16S rRNA gene and draft genome sequences are respectively KR346911 and PZX101000000.1.

Description of *Streptacidiphilus griseoplanus* comb. nov.

Streptomyces gri.se.o.planus. (N.L. adj. *griseus*. grey: L. adj. *planus*, flat, level: N.L. masc. adj.

griseoplanus, flat, gray, referring to restricted, flat, plane growth and grayish spore color *en masse* of the organism).

Basonym: *Streptomyces griseoplanus* Backus et al. 1957

The description is as given for *Streptomyces griseoplanus* Backus *et al.* 1957 with the following addition: strain DSM 4009^T was able to grow in presence of pH5 and up to 1% NaCl. It metabolised dextrin, D-cellobiose, D-fructose, D-galactose, β -gentiobiose, D-glucose, 6, *N*-acetyl-d-glucosamine, D-mannose, D-maltose, D-mannitol, L-rhamnose, D-sorbitol, sodium bromate, D-trehalose and turanose as sugars; α -

and β -hydroxy-butyric acid, α -keto-butyric acid, D-gluconic acid, L-glutamic acid, L-lactic acid, L-malic acid, methyl pyruvate, D-saccharic acid, N-acetyl-neuraminic acid, as organic acids; glycine-proline, l-aspartic acid as amino acids, and degrade gelatine, tween 20, 40, 60, 80, hypoxanthine 0.4%, Tributin 1% and urea. The strain was able to grow in presence of aztreonam, glycerol, nalidixic acid, potassium tellurite and rifamycin SV. The type strain was characterised by LL- A₂pm in the peptidoglycan layer and glucose, mannose (trace) and ribose as cell wall sugars; MK9(H₆) as predominant menaquinone (>25%); *anteiso*-C_{15:0} and C_{16:0} as major fatty acid (15%) and diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), glycerophospholipid (GPL), unknown lipid (L) and aminoglycolipid (AGL), glycolipid (GL), aminolipid (AL) and three unknown phospholipids (PL₁₋₃). The genome size is 8.25 Mb with DNA G+C content of 72.5 mol %. The type strain is DSM 40009^T =NBRC 12779^T =ISP 5009^T =RIA 1046^T =NBRC 12779^T =CBS 505.68^T =IFO 12779^T =ATCC 19766^T =AS 4.1868^T.

Emended description of the genus *Streptoacidiphilus* Kim *et al.* 2003.

The description is as given before by Kim *et al.* 2003 with the following modification. Whole-cell hydrolysates are rich in glucose, mannose and ribose or in galactose and rhamnose. The G+C content is around 70-72.6%. The type species is *Streptoacidiphilus albus* Kim *et al.* 2003.

Acknowledgements

IN is grateful to Newcastle University for a postdoctoral fellowship.

Conflict of interest

The authors declare that they have no conflict of interest. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Ethical statement

This article does not contain any studies inoculating human participants or animals.

References

1. Waksman SA, Henrici AT. The Nomenclature and Classification of the Actinomycetes. *J Bacteriol* 1943;46:337-341.
2. Kim SB, Lonsdale J, Seong CN, Goodfellow M. *Streptacidiphilus* gen. nov., acidophilic actinomycetes with wall chemotype I and emendation of the family *Streptomycetaceae* (Waksman and Henrici (1943)AL) emend. Rainey *et al.* 1997. *Antonie van Leeuwenhoek* 2003;83:107-116.
3. Cho SH, Han JH, Ko HY, Kim SB. *Streptacidiphilus anmyonensis* sp. nov., *Streptacidiphilus rugosus* sp. nov. and *Streptacidiphilus melanogenes* sp. nov., acidophilic actinobacteria isolated from Pinus soils. *Int J Syst Evol Microbiol* 2008;58:1566-1570.
4. Golinska P, Ahmed L, Wang D, Goodfellow M. *Streptacidiphilus durhamensis* sp. nov., isolated from a spruce forest soil. *Antonie van Leeuwenhoek* 2013;104:199-206.
5. Golinska P, Kim BY, Dahm H, Goodfellow M. *Streptacidiphilus hamsterleyensis* sp. nov., isolated from a spruce forest soil. *Antonie van Leeuwenhoek* 2013;104:965-972.
6. Huang Y, Cui Q, Wang L, Rodriguez C, Quintana E, et al. *Streptacidiphilus jiangxiensis* gen. nov., a novel actinomycete isolated from acidic rhizosphere soil in China. *Antonie van Leeuwenhoek* 2004;86:159-165.
7. Song W, Duan L, Jin L, Zhao J, Jiang S et al. *Streptacidiphilus monticola* sp. nov., a novel actinomycete isolated from soil. *Int J Syst Evol Microbiol* 2018;68:1757-1761.
8. Wang L, Huang Y, Liu Z, Goodfellow M, Rodriguez C. *Streptacidiphilus oryzae* sp. nov., an actinomycete isolated from rice-field soil in Thailand. *Int J Syst Evol Microbiol* 2006;56:1257-1261.
9. Golinska P, Dahm H, Goodfellow M. *Streptacidiphilus toruniensis* sp. nov., isolated from a pine forest soil. *Antonie van Leeuwenhoek* 2016;109:1583-1591.
10. Goodfellow M, Williams ST. Ecology of actinomycetes. *Annu Rev Microbiol* 1983;37:189-216.
11. Williams ST, Lanning S, Wellington EMH. Ecology of actinomycetes. In: Goodfellow M, Mordarski M and Williams ST (editors). *The Biology of the Actinomycetes*. London: Academic Press; 1984. pp. 481-528.
12. Williams ST, Khan MR. Antibiotics--a soil microbiologist's viewpoint. *Postepy Hig Med Dosw* 1974;28:395-408.
13. Williams S.T., Robinson C. S. The role of streptomycetes in decomposition of chitin in acidic soils. *J Gen Microbiol* 1981;127:55-63.
14. Williams ST, Flowers TH. The influence of pH on starch hydrolysis by neutrophilic and acidophilic actinomycetes. *Microbios* 1987;20:99-106.
15. Backus EJ, Tresner HD, Campbell TH. The nucleocidin and alazopeptin producing organisms: Two new species of *Streptomyces*. *Antibiotics and Chemotherapy* 1957;7: 532-541.
16. Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 1966;16:313-340.

17. Berd D. Laboratory identification of clinically important aerobic actinomycetes. *Appl Microbiol* 1973;25:665-681.
18. Staneck JL, Roberts GD. Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* 1974;28:226-231.
19. Lechevalier MP, Lechevalier HA. Composition of whole-cell hydrolysates as a criterion in the classification of aerobic actinomycetes. In: Prauser H (editor). *The Actinomycetales*. Jena: Gustav Fischer Verlag; 1970. pp. 311-316.
20. Collins MD. 11 Analysis of isoprenoid quinones. *Meth Microbiol* 1985;18:329-366.
21. Minnikin DE, Goodfellow M. Lipid composition in the classification and identification of nocardiae and related taxa. In: Goodfellow M, Brownell GH and Serrano JA (editors). *The Biology of the Nocardiae*. London: Academic Press; 1976. pp. 160-219.
22. Kroppenstedt RM, Goodfellow M. The family *Thermomonosporaceae*: *Actinocorallia*, *Actinomadura*, *Spirillispota* and *Thermomonospora*. In: Dworkin M, Falkow K, Schleifer KH and Stackebrandt E (editors). *The Prokaryotes Archaea and Bacteria: Firmicutes, Actinomycetes*, 3rd edn, vol. 3. New York: Springer; 2006. pp. 682-724.
23. Miller LT. Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* 1982;16:584-586.
24. Kuykendall LD, Roy MA, O'Neill JJ, Devine TE. Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Evol Microbiol* 1988;38:358-361.
25. Sasser MJ. Identification of bacteria by gas chromatography of cellular fatty acids. Technical Note 101, Microbial ID. USA: Inc, Newark, Del, 1990.
26. Kämpfer P. Genus *incertae sedis* II. *Streptacidiphilus* Kim, Lonsdale, Seong and Goodfellow 2003a, 1219VP (Effective publication: Kim, Lonsdale, Seong and Goodfellow 2003b, 115.). In: Goodfellow M, Kämpfer P, Busse HJ, Trujillo ME, Suzuki K-I, Ludwig W and Whitman WB (editors). *Bergey's Manual of Systematic Bacteriology* 2nd edn; vol 5. The *Actinobacteria*, Part B. New York: Springer; 2012. pp.1777-1805.
27. Groth I, Rodríguez C, Schütze B, Schmitz P, Leistner E. et al. Five novel *Kitasatospora* species from soil: *Kitasatospora arboriphila* sp. nov., *K. gansuensis* sp. nov., *K. nipponensis* sp. nov., *K. paranensis* sp. nov. and *K. terrestris* sp. nov. *Int J Syst Evol Microbiol* 2004;54:2121-2129.
28. Duggar BM. Aureomycin; a product of the continuing search for new antibiotics. *Ann N Y Acad Sci* 1948; 51:177-181.
29. Labeda DP, Dunlap CA, Rong X, Huang Y, Doroghazi JR, et al. Phylogenetic relationships in the family *Streptomycetaceae* using multi-locus sequence analysis. *Antonie van Leeuwenhoek* 2017;110:563-583.
30. Baldacci E, Grein A. *Streptomyces avellaneus* and *Streptomyces libani*: two new species characterized by a hazel-nut brown (avellaneus) aerial mycelium. *Giornale di Microbiologia* 1966;14:185-198.
31. Liu Z, Rodríguez C, Wang L, Cui Q, Huang Y, et al. *Kitasatospora viridis* sp. nov., a novel actinomycete from soil. *Int J Syst Evol Microbiol* 2005;55:707-711.
32. Groth I, Schütze B, Boettcher T, Pullen CB, Rodriguez C, et al. *Kitasatospora putterlickiae* sp. nov., isolated from rhizosphere soil, transfer of *Streptomyces kifunensis* to the genus *Kitasatospora* as *Kitasatospora viridis* comb. nov., and emended description of *Streptomyces aureofaciens* Duggar 1948. *Int J Syst Evol Microbiol* 2003; 53:2033-2040.
33. Vaas LA, Sikorski J, Michael V, Göker M, Klenk HP. Visualization and curve-parameter estimation strategies for efficient exploration of phenotype microarray kinetics. *PLoS One* 2012;7:e34846.
34. Vaas LA, Sikorski J, Hofner B, Fiebig A, Buddhuhs N, et al. opm: an R package for analysing OmniLog(R) phenotype microarray data. *Bioinformatics* 2013;29:1823-1824.
35. Weyant RS, Moss CW, Weaver RE, Hollis DG, Jordan JJ, et al. Identification of unusual pathogenic gram-negative aerobic and facultatively anaerobic bacteria. 2nd edn Baltimore, MD: Williams & Wilkins; 1966.
36. Lasker BA, Bell M, Klenk HP, Sproer C, Schumann C, et al. *Nocardia vulneris* sp. nov., isolated from wounds of human patients in North America. *Antonie van Leeuwenhoek* 2014;106:543-553.
37. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, et al. Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613-1617.

38. Meier-Kolthoff JP, Göker M, Sproer C, Klenk HP. When should a DDH experiment be mandatory in microbial taxonomy? *Arch Microbiol* 2013;195:413-418.
39. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
40. Meier-Kolthoff JP, Hahnke RL, Petersen J, Scheuner C, Michael V, et al. Complete genome sequence of DSM 30083(T), the type strain (U5/41(T)) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. *Stand Genomic Sci* 2014;9:2.
41. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004;32:1792-1797.
42. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368-376.
43. Kluge AG, Farris FS. Quantitative phyletics and the evolution of anurans. *Syst Zool* 1969;18:1-32.
44. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312-1313.
45. Goloboff PA, Farris JS, Nixon KC. TNT, a free program for phylogenetic analysis. *Cladistics* 2008;24:774-786.
46. Pattengale ND, Alipour M, Bininda-Emonds OR, Moret BM, Stamatakis A. How many bootstrap replicates are necessary? *J Comput Biol* 2010;17:337-354.
47. Swofford DL. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0. Sunderland: Sinauer Associates; 2002.
48. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406-425.
49. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol* 2016;33:1870-1874.
50. Nouioui I, Carro L, Garcia-Lopez M, Meier-Kolthoff JP, Klenk HP, et al. Genome-based taxonomic classification of the phylum Actinobacteria. *Front Microbiol* 2018;submitted
51. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114-2120.
52. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455-477.
53. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 2009;25:2078-2079.
54. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv* 2013;3:13033997.
55. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 2014;9:e112963.
56. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81-91.
57. Mesbah M, Premachandran U, Whitman WB. Precise Measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* 1989;39:159-167.
58. Kim M, Oh HS, Park SC, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 2014;64:346-351.
59. Wayne LG, Brenner BJ, Colwell RR, Grimont PAD, Kandler O, et al. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst bacteriol* 1987;37:463-464.
60. Komaki H, Ichikawa N, Hosoyama A, Fujita N, Igarashi Y. Draft genome sequence of *Streptomyces* sp. TP-A0882 reveals putative butyrolactol biosynthetic pathway. *FEMS Microbiol Lett* 2015;362.
61. Pope MK, Green B, Westpheling J. The *bldB* gene encodes a small protein required for morphogenesis, antibiotic production, and catabolite control in *Streptomyces coelicolor*. *J Bacteriol* 1998;180:1556-1562.
62. Labeda DP, Dunlap CA, Rong X, Huang Y, Doroghazi JR, et al. Phylogenetic relationships in the family *Streptomycetaceae* using multi-locus sequence analysis. *Antonie van Leeuwenhoek* 2017;110:563-583.

63. Hanage WP, Fraser C, Spratt BG. Fuzzy species among recombinogenic bacteria. *BMC Biol* 2005;3:6.

Figure Legends

Fig 1. Maximum-likelihood phylogenetic tree, based on the 16S rRNA gene sequences showing the phylogenetic relationship of strain 15-057A^T with its closely related species. Bootstrap percentages based on 1,000 resampled data sets; only values $\geq 60\%$ are shown. The numbers above the branches are bootstrap values from maximum-likelihood (left) and maximum parsimony (right). Bar, 0.007 substitutions per nucleotide position.